English Translation of JP-A-11-228685

[Title of the Invention]

CARBOXYL GROUP-CONTAINING POLYOXYALKYLENE COMPOUND

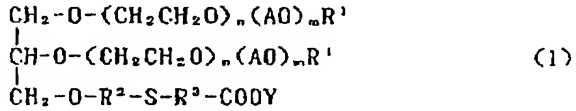
[Abstract]

[Problem]

To provide a carboxyl group-containing polyoxyalkylene compound which can easily react with an amino group or a hydroxyl group of polypeptides, physiologically active proteins, enzymes, and the like and can achieve performance characteristics such as reduction of antigenicity of the compounds, stabilization thereof, and extension of the residence time thereof in a body (blood) and which has a low toxicity and results in small formation of by-products.

[Means for Solution]

A carboxyl group-containing polyoxyalkylene compound represented by the formula (1):



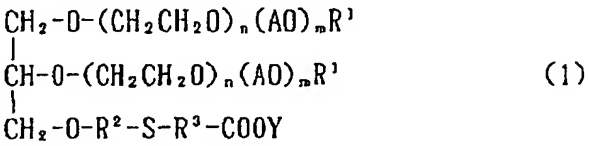
(1)

wherein R^1 is a hydrogen atom, a hydrocarbon group having 1 to 24 carbon atoms or an acyl group having 1 to 24 carbon atoms; R^2 is a hydrocarbon group having 3 to 4

carbon atoms; R^3 is a hydrocarbon group having 1 to 10 carbon atoms; AO is an oxyalkylene group having 3 to 4 carbon atoms; Y represents a hydrogen atom or an active group; n means an average added mole number of the oxyethylene group and is from 1 to 1,000; m means an average added mole number of the oxyalkylene group having 3 to 4 carbon atoms; n/(n+m) is 0.8 or more; and an addition mode of the oxyethylene group and the oxyalkylene group having 3 to 4 carbon atoms may be a block form or a random form.

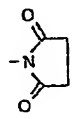
[Claims]

[Claim 1] A carboxyl group-containing polyoxyalkylene compound represented by the formula (1):

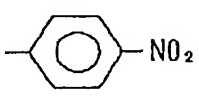


(1)

wherein R^1 is a hydrogen atom, a hydrocarbon group having 1 to 24 carbon atoms or an acyl group having 1 to 24 carbon atoms; R^2 is a hydrocarbon group having 3 to 4 carbon atoms; R^3 is a hydrocarbon group having 1 to 10 carbon atoms; AO is an oxyalkylene group having 3 to 4 carbon atoms; Y represents a hydrogen atom or an active group represented by the formula (2) or (3); n means an average added mole number of the oxyethylene group and is from 1 to 1,000; m means an average added mole number of the oxyalkylene group having 3 to 4 carbon atoms; n/(n+m) is 0.8 or more; and an addition mode of the oxyethylene group and the oxyalkylene group having 3 to 4 carbon atoms may be a block form or a random form.



(2)



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[Detailed Description of the Invention]

[0001]

[Technical Field to which the Invention Belongs]

The present invention relates to a polyoxyalkylene compound having polyoxyalkylene chains at α- and β-positions of glycerin and having a carboxyl group or an activated carboxyl group at γ-position thereof. More specifically, it relates to a polyoxyalkylene compound having a terminal carboxyl group, which is mainly used in pharmaceutical applications, e.g., polyoxyalkylene-modification of polypeptides, physiologically active proteins, enzymes, and the like and modification of polyoxyalkylene groups in drug delivery systems such as liposomes, polymer micelles, and the like.

[0002]

[Prior Art]

Heretofore, compounds wherein a terminal hydroxyl group of polyoxyalkylene glycol is replaced by a carboxyl group are described as a lubricating oil (JP-B-63-4877) or as an additive for synthetic resins (JP-A-63-182343) and have been widely utilized. Recently, polyoxyalkylene compounds have attracted attention as important carriers for drug delivery systems and studies have also been actively carried out on compounds wherein an amino group or a carboxyl group is introduced into polyoxyalkylene

compounds. Of these, as a compound having two polyoxyalkylene chains, there is known 2,4-bis(O-methoxypolyethylene glycol)-6-chloro-S-triazine wherein a triazine ring intervenes (hereinafter referred to as "activated PEG2") as shown in JP-A-3-72469. Moreover, a polyoxyalkylene compound having a large number of amino groups at side chains of a polyoxyalkylene group is also known (JP-A-8-48764).

[0003]

[Problems that the Invention is to Solve]

Particularly, in a compound or a drug modified with a polyoxyalkylene compound (e.g., a protein, a physiologically active compound, DNA, etc.), or a drug delivery system utilizing the modification, there are obtained effects such as (1) reduction of antigenicity (imunoreactivity), (2) increase of stability as the compound or drug, and (3) extension of the residence time in a body. However, with regard to these conventional carboxyl group-containing polyoxyalkylene compounds, for example, in the case of single-chain polyoxyalkylene compounds containing a terminal carboxyl group, there frequently exist cases that, when objective substances are modified with them, the performance characteristics such as reduction of antigenicity and stabilization of the objective substances inherent to polyoxyalkylene are not

sufficiently achieved since they are single-chain compounds. Moreover, since the aforementioned activated PEG2 possesses a triazine ring, there is a possibility of appearance of toxicity when it is administered into a body as a pharmaceutical. Furthermore, with regard to the compound having a number of carboxyl groups at side chains of a polyoxyalkylene skeleton, the modification reaction is difficult to control because there exist a large number of reaction sites and hence it is difficult to obtain a single compound.

[0004]

An object of the invention is to provide a carboxyl group-containing polyoxyalkylene compound which is used for modification of a phospholipid for the purpose of reduction of antigenicity of a compound or a drug, stabilization thereof, and extension of the residence time thereof in a body and wherein the modified compound or drug has a low toxicity and formation of by-products is small.

[0005]

[Means for Solving the problems]

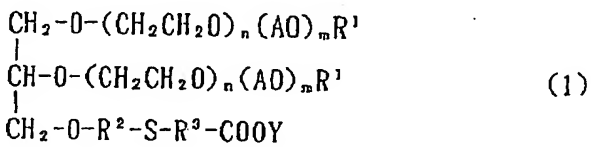
As a result of extensive studies for solving the above problems, the present inventors have found that the above object can be achieved by a polyoxyalkylene compound having polyoxyalkylene chains at α- and β- positions of

glycerin and having a carboxyl group or a carboxyl group activated with N-hydroxysuccinimide at γ-position thereof, and thus they have accomplished the invention.

[0006]

Namely, the invention is a carboxyl group-containing polyoxyalkylene compound represented by the formula (1):

[0007]

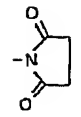


(1)

[0008]

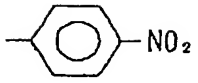
wherein R^1 is a hydrogen atom, a hydrocarbon group having 1 to 24 carbon atoms or an acyl group having 1 to 24 carbon atoms; R^2 is a hydrocarbon group having 3 to 4 carbon atoms; R^3 is a hydrocarbon group having 1 to 10 carbon atoms; AO is an oxyalkylene group having 3 to 4 carbon atoms; Y represents a hydrogen atom or an active group represented by the formula (2) or (3); n means an average added mole number of the oxyethylene group and is from 1 to 1,000; m means an average added mole number of the oxyalkylene group having 3 to 4 carbon atoms; n/(n+m) is 0.8 or more; and an addition mode of the oxyethylene group and the oxyalkylene group having 3 to 4 carbon atoms may be a block form or a random form.

[0009]



(2)

[0010]



(3)

[0011]

[Mode for Carrying Out the Invention]

In the formula (1), the hydrocarbon group having 1 to 24 carbon atoms represented by R^1 includes linear or branched alkyl groups such as a methyl group, an ethyl group, a propyl group, an isopropyl group, a butyl group, an isobutyl group, a tert-butyl group, a pentyl group, an isopentyl group, a hexyl group, an isohexyl group, a 2-ethylhexyl group, an octyl group, an isononyl group, a decyl group, a dodecyl group, an isotridecyl group, a tetradecyl group, a hexadecyl group, an isocetyl group, an octadecyl group, an isostearyl group, an octyldodecyl group, a docosyl group, and a decyltetradecyl group, as aliphatic hydrocarbon groups; and aryl groups such as a butylphenyl group, a dibutylphenyl group, an octylphenyl group, a dinonylphenyl group, and an α-methylbenzylphenyl group, aralkyl groups such as a benzyl group, and a cresyl

group, as aromatic hydrocarbon groups.

[0012]

Moreover, the acyl group having 1 to 24 carbon atoms includes acyl groups derived from acetic acid, propionic acid, butyric acid, isobutyric acid, caprylic acid, 2-ethylhexanoic acid, isononanoic acid, capric acid, lauric acid, myristic acid, palmitic acid, isopalmitic acid, stearic acid, isostearic acid, arachidic acid, behenic acid, palmitoleic acid, benzoic acid, hydroxybenzoic acid, cinnamic acid, gallic acid, and the like. Of these, as R^1, a hydrogen atom and a linear alkyl group having 1 to 4 carbon atoms are preferred. Incidentally, two R^1 are present in the formula (1), but these may be the same or different from each other.

[0013]

The hydrocarbon group having 3 to 4 carbon atoms represented by R^2 includes groups derived from hydrocarbon groups having a polymerizable unsaturated group, preferably groups derived from hydrocarbon groups having a double bond, e.g., an allyl group and a methallyl group, linear or branched alkylene groups such as trimethylene group and isobutylene group, and the like.

[0014]

The hydrocarbon group having 1 to 10 carbon atoms represented by R^3 includes linear or branched alkylene

groups such as a methylene group, an ethylene group, a propylene group, and a trimethylene group, and divalent aromatic hydrocarbon groups such as a phenylene group and a benzyl group. Of these, a methylene group and an ethylene group are preferred.

[0015]

The alkylene part of the oxyalkylene group having 3 to 4 carbon atoms represented by AO may be linear or branched and examples of such an oxyalkylene group include an oxypropylene group, an oxytrimethylene group, an oxybutylene group, and an oxytetramethylene group.

[0016]

Y is a hydrogen atom or an active group represented by the formula (2) or (3), but the active group represented by the formula (2) or (3) is preferred in view of reactivity with objective substances.

[0017]

n means an average added mole number of the oxyethylene group and is from 1 to 1,000; m means an average added mole number of the oxyalkylene group having 3 to 4 carbon atoms; and n/(n+m) is 0.8 or more, and an addition mode of the oxyethylene group and the oxyalkylene group having 3 to 4 carbon atoms may be a block form or a random form.

[0018]

The carboxyl group-containing polyoxyalkylene compound of the invention represented by the formula (1) can be produced, for example, as follows. First, ethylene oxide alone or ethylene oxide and an alkylene oxide having 3 to 4 carbon atoms are added to a compound represented by the formula (4):

[0019]

(4)

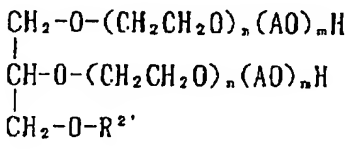
[0020]

wherein $R^{2'}$ represents a hydrocarbon group having a polymerizable unsaturated group, preferably a double bond-containing alkyl group having 3 to 4 carbon atoms, such as an allyl group or a methallyl group. At this time, after the addition of ethylene oxide to the compound (2), the alkylene oxide having 3 to 4 carbon atoms may be added, or ethylene oxide and the alkylene oxide having 3 to 4 carbon atoms may be mixed and the addition reaction may be effected at a time. The ratio of the added mole number of ethylene oxide to that of the alkylene oxide having 3 to 4 carbon atoms is determined so that the oxyethylene group should account for 80% or more in order to maintain hydrophilicity of the whole oxyalkylene chain.

[0021]

Specifically, the compound (4) is charged into a reactor, substitution with nitrogen is conducted, and an alkylene oxide (ethylene oxide only or a mixture of ethylene oxide and an alkylene oxide having 3 to 4 carbon atoms) is charged with pressure at 100 to 140°C, followed by reacting them. After the reaction, unreacted alkylene oxide is removed under reduced pressure, the reaction mixture is cooled to 80°C, neutralized by adding an acid such as phosphoric acid or hydrochloric acid, and dehydrated and filtrated to obtain a compound represented by the formula (5'):

[0022]

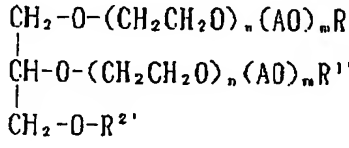


(5')

[0023]

wherein the symbols are as described above. If necessary, by introduction of a hydrocarbon group, e.g., alkylation or acylation of the terminal hydroxyl group, the compound is converted into a compound represented by the formula (5''):

[0024]



(5'')

[0025]

wherein $R^{1'}$ represents a hydrocarbon group having 1 to 24 carbon atoms or an acyl group having 1 to 24 carbon atoms, and the other symbols are as described above.

[0026]

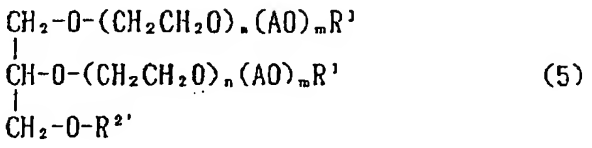
For example, in the alkylation reaction, an alkylating agent such as an alkyl chloride (halogenated alkyl) or an alkenyl chloride having a hydrocarbon group represented by R^1 is added in an amount of 1.1 to 3.0 molar equivalents to a hydroxyl group of the compound (5') and the reaction is conducted at 90 to 120°C for 2 to 5 hours, followed by washing with water, removal of unreacted matter, neutralization, dehydration, and filtration. In the acylation reaction, an acylating agent such as an acyl halide or a carboxylic anhydride having an acyl group represented by R^1 is added in an amount of 1.1 to 2.0 molar equivalents to a hydroxyl group of the compound (5') and a dehydration-condensation reaction is conducted at 110 to 140°C for 9 hours in the presence of p-toluenesulfonic acid, followed by treatment with an adsorbent, dehydration, and filtration. In the case that a compound wherein R^1 in the above halide or carboxylic anhydride is an aromatic hydrocarbon group is used, an aromatic hydrocarbon group is introduced. The reaction conditions in this case are in accordance with those in

the above alkylation and acylation.

[0027]

To a thus obtained compound represented by the formula (5):

[0028]



(5)

[0029]

wherein respective symbols are as described above,

a compound represented by the formula (6):

$HS-R^3-COOH$ (6)

wherein R^3 is as described above,

is added in an amount of 1.5 to 10 molar equivalents to the allyl group or methallyl group in the compound (5) and the reaction is conducted in an alcohol such as methanol or ethanol at 30 to 40°C for 3 to 7 hours to introduce a carboxyl group. After completion of the reaction, the alcohol is evaporated. The reaction mixture is dissolved in a solvent such as chloroform or dichloromethane and then washed with water to remove the unreacted compound (6). Then, after removal of the solvent by evaporation, filtration is conducted to obtain the compound of the formula (1) wherein Y is a hydrogen atom.

[0030]

Thereafter, for example, the compound is reacted with N-hydroxysuccinimide or p-nitrophenol at 30 to 40°C in a solvent such as dimethylformamide, chloroform, or toluene in the presence of dicyclohexylcarbodiimide and, after filtration, crystallization is conducted from isopropyl alcohol or hexane to form a compound of the formula (1) in which the carboxyl group is activated, wherein Y is an activated group of the formula (2) or (3).

[0031]

The carboxyl group-containing polyoxyalkylene compound of the invention is thought to be used for (1) modification of antitumor proteins such as asparaginase and arginase, (2) modification of metabolic disorder enzymes such as adenosine deaminase, insulin, and uricase, (3) modification of antigen proteins such as immunoglobulin and serum albumin, (4) modification of antiinflammatory enzymes such as catalase and superoxide dismutase, and (5) modification of blood component proteins such as albumin and granulocyte colony stimulator. Moreover, as utilization for drug delivery systems, it is thought to use for chemical modification of a phospholipid including an anticancer agent such as adriamycin or cisplatin therein, which is a basal material for fat emulsions or liposomes. In both cases, by the

modification with the polyoxyalkylene group, effects such as improvement of immunogenicity, stabilization of drugs, and extension of the residence time in a blood are expected.

[0032]

[Examples]

The following will describe the present invention in further detail with reference to Examples.

Production Example 1

Into a 5 liter-volume autoclave were charged 66 g (0.5 mol) of glycerin monoallyl ether and 1 g of potassium hydroxide. The atmosphere of the system was substituted with nitrogen gas, followed by temperature elevation to 120°C. Then, 2440 g (55 mol) of ethylene oxide was charged with pressure, followed by 1 hour of reaction at 130±5°C. Then, unreacted ethylene oxide was removed under reduced pressure (200 mmHg, 0.5 hour) while nitrogen gas was allowed to pass through and the whole was cooled to 80°C. Thereafter, pH was adjusted to 7.0 with a 10 wt% hydrochloric acid aqueous solution and dehydration was conducted at 100±5°C under 100 mmHg for 1 hour. Then, the reaction mixture was cooled to 80°C and the precipitated salt was filtrated off to obtain 2380 g of a compound.

[0033]

The hydroxyl value of the resulting compound was

22.4 (calculated value: 23.0) and the degree of unsaturation was 0.19 (calculated value: 0.2). In this connection, the hydroxyl value was measured in accordance with the method of JIS K-15576.4 (1970) and the degree of unsaturation was measured in accordance with the method of JIS K-15576.7 (1970).

[0034]

An infrared absorption spectrum of the compound is shown in Fig. 1. Analytical results of gel permeation chromatography (hereinafter referred to GPC) are shown in Fig. 2 and Table 1. Analytical conditions of GPC are as follows.

GPC system: SYSTEM-11 (Syowa Denko K.K.)

GPC column: SHODEX KF-804L ×3

Developing liquid: THF

Flow rate: 1 ml/min

Sample concentration: 0.15 wt%

Column oven temperature: 40°C

[0035]

[Table 1]

Peak information	Time (minute)	Molecular weight	Height
Start	19.9	20759	2
Top	22.339	5193	34156
End	25.3	1114	0
Number-average molecular weight (MN)		5131	
Weight-average molecular weight (MW)		5268	

[0036]

Results of ^{1}H-NMR spectrum are as follows.

^{1}H-NMR (δ (ppm), CDCl/TMS)

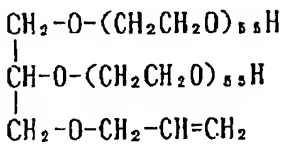
δ=5.2 ppm (C=C<u>H$_2$</u>)

δ=5.9 ppm (-C<u>H</u>=)

[0037]

From the starting materials, the reaction conditions, and the above analytical values, the resulting compound was deduced to be a compound represented by the formula (7):

[0038]



(7)

[0039]

(molecular weight: 5009).

[0040]

Production Example 2

Into a 5 liter-volume autoclave were charged 66 g (0.5 mol) of glycerin monoallyl ether and 0.6 g of potassium hydroxide. The atmosphere of the system was substituted with nitrogen gas, followed by temperature elevation to 100°C. Then, 1340 g (30 mol) of ethylene oxide and 110 g (2 mol) of propylene oxide were weighed into a weighing vessel and were mixed until they formed a homogeneous mixture. The mixture of ethylene oxide and propylene oxide was charged with pressure from the weighing vessel under conditions of 110±5°C and 10 kg/cm^2 or lower over the period of 8 hours. After the charging with pressure, reaction was conducted for 1 hour. Then, unreacted ethylene oxide and propylene oxide were removed under reduced pressure of 200 mmHg for 30 minutes while nitrogen gas was allowed to pass through, followed by cooling to 80°C. Thereafter, pH was adjusted to 7.0 with a 10 wt% hydrochloric acid aqueous solution and dehydration was conducted under conditions of 100±5°C and 100 mmHg for 1 hour. Then, the reaction mixture was cooled to 80°C and the precipitated salt was filtrated off to obtain 1440 g of a compound.

[0041]

The hydroxyl value of the resulting compound was 36.4 (calculated value: 36.2) and the degree of unsaturation was 0.30 (calculated value: 0.32). In this connection, the hydroxyl value and the degree of unsaturation were measured as in Production Example 1. Analytical results of GPC are shown in Fig. 3 and Table 2. Analytical conditions of GPC were as in Production Example 1.

[0042]

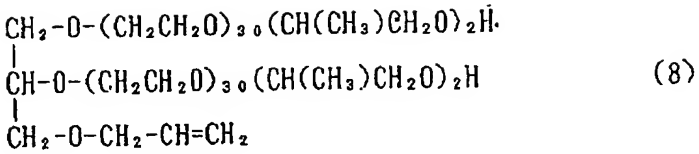
[Table 2]

Peak information	Time (minute)	Molecular weight	Height
Start	21.2	9539	1
Top	23.341	3082	24069
End	25.6	953	36
Number-average molecular weight (MN)		2969	
Weight-average molecular weight (MW)		3061	

[0043]

From the starting materials, the reaction conditions, and the above analytical values, the resulting compound was deduced to be a compound represented by the formula (8):

[0044]



(8)

[0045]

(molecular weight: 3082).

[0046]

Production Example 3

Into a 5 liter-volume autoclave were charged 1000 g (0.32 mol) of the compound of the formula (8) obtained in Production Example 2 and 150 g of potassium hydroxide. The atmosphere of the system was substituted with nitrogen gas, followed by temperature elevation to 100°C. Then, 43.5 g (0.84 mol) of methyl chloride was charged thereto under a condition of 100±5°C. After 4 hours of reaction, the whole was cooled to 80°C and unreacted methyl chloride was removed under reduced pressure (200 mmHg or lower) for 0.5 hour while nitrogen gas was allowed to pass through. Then, after 500 g of water was added into the system and the whole was mixed, the mixture was left on standing to be allowed to separate into layers and excess alkali matter of the lower layer was removed. Thereafter, pH was adjusted to 7.0 with a 10 wt% hydrochloric acid aqueous solution and dehydration was conducted under conditions of

100±5°C and 100 mmHg for 1 hour. Then, the reaction mixture was cooled to 80°C and the precipitated salt was filtrated off to obtain 955 g of a compound.

[0047]

The hydroxyl value of the resulting compound was 0.04 (calculated value: 0) and the degree of unsaturation was 0.29 (calculated value: 0.32). In this connection, the hydroxyl value and the degree of unsaturation were measured as in Production Example 1. Analytical results of GPC are shown in Fig. 4 and Table 3. Analytical conditions of GPC were as in Production Example 1.

[0048]

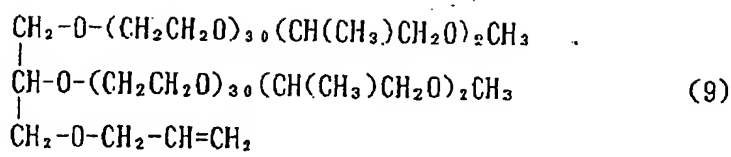
[Table 3]

Peak information	Time (minute)	Molecular weight	Height
Start	21.3	9029	2
Top	23.327	3106	25082
End	25.4	1057	28
Number-average molecular weight (MN)		2987	
Weight-average molecular weight (MW)		3075	

[0049]

From the starting materials, the reaction conditions, and the above analytical values, the resulting compound was deduced to be a compound represented by the formula (9):

[0050]



(9)

[0051]

(molecular weight: 3110).

[0052]

Example 1

In a four-neck flask was placed 37 g (0.4 mol) of mercaptoacetic acid ($HSCH_2COOH$) as a compound of the formula (6), and the temperature was kept at 35±5°C under stirring. Then, 500 g (0.1 mol) of the compound of the formula (7) synthesized in Production Example 1 was dissolved in 500 g of methanol and the resulting solution was added dropwise to the four-necked flask through a dropping funnel over the period of 5 hours. After completion of the addition of the whole amount thereof, the whole was kept at 40±5°C for further 5 hours to continue the reaction. Then, methanol was removed by evaporation at 60±10°C under reduced pressure of 200 mmHg or lower and then the reaction mixture was again dissolved in 1000 g of chloroform. Then, the whole amount thereof was transferred into a separating funnel and was washed with 1 liter of saturated saline three times to remove

unreacted mercaptoacetic acid. Then, chloroform and water were removed by evaporation at 110±10°C under a nitrogen atmosphere under reduced pressure of 50 mmHg or lower and the precipitated sodium chloride was removed by filtration, whereby 472 g of a compound (molecular weight: 5054) was obtained.

[0053]

The acid value of the resulting compound was 11.6 (calculated value: 11.2) and the degree of unsaturation was 0.01 (calculated value: 0). In this connection, the acid value was measured in accordance with the method of JIS K-1557, 6.6 (1970) and the degree of unsaturation was measured as in Production Example 1. An infrared absorption spectrum is shown in Fig. 5. Results of ^{1}H-NMR spectrum are as follows.

^{1}H-NMR (δ (ppm), CDCl/TMS)

δ=1.85 ppm (-O-$CH_2C\underline{H_2}CH_2$-S-CH_2COOH)

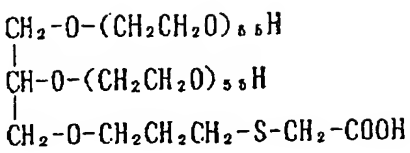
δ=2.75 ppm (-O-$CH_2CH_2C\underline{H_2}$-S-CH_2COOH)

δ=3.2 ppm (-O-$CH_2CH_2CH_2$-S-$C\underline{H_2}COOH$)

[0054]

From the starting materials, the reaction conditions, and the above analytical values, the resulting compound was deduced to be a compound represented by the formula (10):

[0055]



(10)

[0056]

[0057]

Example 2

In a four-neck flask was placed 59 g (0.64 mol) of mercaptoacetic acid ($HSCH_2COOH$) as a compound of the formula (6), and the temperature was kept at 35±5°C under stirring. Then, 500 g (0.16 mol) of the compound of the formula (9) synthesized in Production Example 3 was dissolved in 500 g of methanol and the resulting solution was added dropwise to the four-necked flask through a dropping funnel over the period of 5 hours. After completion of the dropwise addition of the whole amount thereof, the mixture was kept at 40±5°C for further 5 hours to continue the reaction. Then, methanol was removed by evaporation at 60±10°C under reduced pressure of 200 mmHg or lower and then the residue was again dissolved in 1000 g of chloroform. Then, the whole amount thereof was transferred into a separating funnel and was washed with 1 liter of saturated saline three times to remove unreacted mercaptoacetic acid. Then, chloroform and water were removed by evaporation at 110±10°C under a

nitrogen atmosphere under reduced pressure of 50 mmHg or lower and the precipitated sodium chloride was removed by filtration, whereby 470 g of a compound (molecular weight: 3169) was obtained.

[0058]

The acid value of the resulting compound was 17.7 (calculated value: 17.5) and the degree of unsaturation was 0.01 (calculated value: 0). In this connection, the acid value was measured as in Example 1 and the degree of unsaturation was measured as in Production Example 1.

[0059]

Results of ^{1}H-NMR spectrum are as follows.

^{1}H-NMR (δ (ppm), CDCl/TMS)

δ=1.85 ppm (-O-$CH_2\underline{CH_2}CH_2$-S-CH_2COOH)

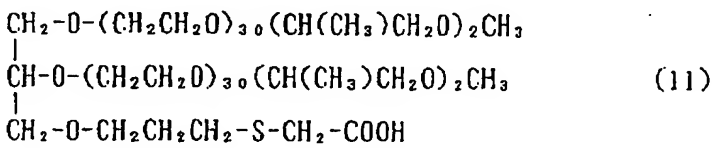
δ=2.75 ppm (-O-$CH_2CH_2\underline{CH_2}$-S-CH_2COOH)

δ=3.2 ppm (-O-$CH_2CH_2CH_2$-S-$\underline{CH_2}$COOH)

[0060]

From the starting materials, the reaction conditions, and the above analytical values, the resulting compound was deduced to be a compound represented by the formula (11):

[0061]



(11)

[0062]

[0063]

Example 3

In a four-neck flask was placed 42 g (0.4 mol) of 3-mercaptopropionic acid ($HSCH_2CH_2COOH$) as a compound of the formula (6), and the temperature was kept at 35±5°C under stirring. Then, 500 g (0.1 mol) of the compound of the formula (7) synthesized in Production Example 1 was dissolved in 500 g of methanol and the resulting solution was added dropwise to the four-necked flask through a dropping funnel over the period of 5 hours. After completion of the dropwise addition of the whole amount thereof, the whole was kept at 40±5°C for further 5 hours to continue the reaction. Then, methanol was removed by evaporation at 60±10°C under reduced pressure of 200 mmHg or lower and then the residue was again dissolved in 1000 g of chloroform. Then, the whole amount thereof was transferred into a separating funnel and was washed with 1 liter of saturated saline three times to remove unreacted mercaptopropionic acid. Then, chloroform and water were removed by evaporation at 110±10°C under a nitrogen atmosphere under reduced pressure of 50 mmHg or lower and the precipitated sodium chloride was removed by filtration, whereby 472 g of a compound (molecular weight: 4878) was obtained.

[0064]

The acid value of the resulting compound was 11.5 (calculated value: 11.2) and the degree of unsaturation was 0.01 (calculated value: 0). In this connection, the acid value was measured as in Example 1 and the degree of unsaturation was measured as in Production Example 1.

[0065]

Results of ^{1}H-NMR spectrum are as follows.

^{1}H-NMR (δ (ppm), CDCl/TMS)

δ=1.85 ppm (-O-$CH_2\underline{CH_2}CH_2$-S-CH_2CH_2-COOH)

δ=2.75 ppm (-O-$CH_2CH_2\underline{CH_2}$-S-CH_2CH_2-COOH)

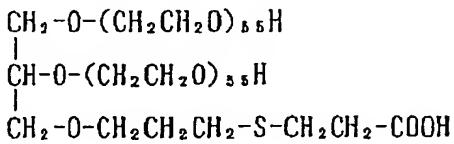
δ=2.85 ppm (-O-$CH_2CH_2CH_2$-S-$\underline{CH_2}CH_2$-COOH)

δ=2.81 ppm (-O-$CH_2CH_2CH_2$-S-$CH_2\underline{CH_2}$-COOH)

[0066]

From the starting materials, the reaction conditions, and the above analytical values, the resulting compound was deduced to be a compound represented by the formula (12):

[0067]



(12)

[0068]

[0069]

Example 4

In a four-neck flask were placed 300 g (0.06 mol) of the compound of the formula (10) obtained in Production Example 1 and 450 g of dimethylformamide, and the temperature was elevated to 50°C under stirring to dissolve them. Then, the temperature was cooled to 35±5°C and 8.3 g (0.07 mol) of N-hydroxysuccinimide and 14.7 g (0.07 mol) of dicyclohexylcarbodiimide were added thereto, followed by 2 hours of reaction. After completion of the reaction, filtration under pressure was conducted and 5 liters of isopropyl alcohol cooled to -10°C was added to the resulting solution, followed by 0.5 hour of stirring at room temperature to precipitate crystals of a polyoxyalkylene compound. The resulting crystals were collected by filtration under reduced pressure and then, after cooled again to -10°C, 5 liters of isopropyl alcohol was added, followed by 0.5 hours of washing. The crystals were again collected by filtration under reduced pressure and then 10 liters of hexane was added to wash the crystals. Finally, the resulting crystals were vacuum-dried at 35°C under 50 mmHg or lower for 4 hours using a vacuum dryer, whereby 245 g of a compound (molecular weight: 5147) was obtained.

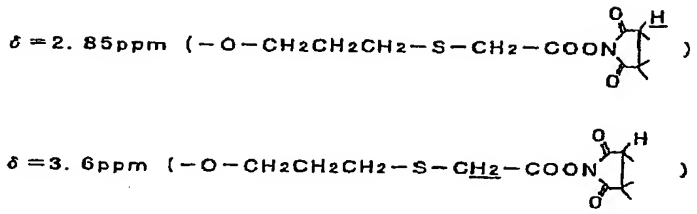
[0070]

An infrared absorption spectrum of the compound is

shown in Fig. 6. Results of ^{1}H-NMR spectrum are as follows.

^{1}H-NMR (δ (ppm), CDCl/TMS)

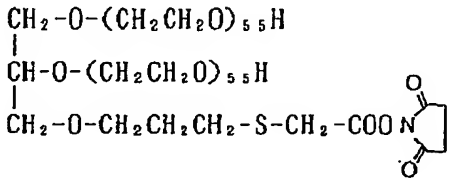
[0071]



[0072]

From the starting materials, the reaction conditions, and the above analytical values, the resulting compound was deduced to be a compound represented by the formula (13):

[0073]



(13)

[0074]

[0075]

Test Example 1

To 2 ml of 0.1M borate buffer (pH 10) containing 10 mg of L-asparaginase was added the compound of the formula (13) obtained in Example 3 in an amount of 15 molar equivalents to an amino group in the asparaginase molecule,

followed by 1 hour of reaction at 370C. Purification was conducted in a usual manner to obtain a modified asparaginase as a white powder. The molecular weight thereof was 400,000 and, as a result of analysis of the amino group, 52 groups were combined, so that the molecular weight was almost coincident with the total value of molecular weight of the added part of 52 × 5150 = ca. 267000 and the molecular weight of asparaginase of 134000. The binding ability with antibody was completely disappeared in this substance but it maintained enzymatic activity at the rate of 37% in Method A and 41% in Method B. These results are shown in Table 4.

[0076]

In this connection, the number of amino groups combined in the asparaginase molecule was measured with trinitrobenzenesulfonic acid. Moreover, the enzymatic activity was measured by a method of spectroscopically measuring a changed amount of NAD+ involved in malic acid formation using L-glutamic acid-oxalacetic acid transaminase (Method A) and by a method of coloring, with ferric chloride, the formation of aspartate hydroxamate by the above enzyme in the co-presence of aspartic acid and hydroxyamine (Method B). Furthermore, the antigenicity was measured by a method of measuring an amount of precipitate formed by an antigen-antibody reaction using

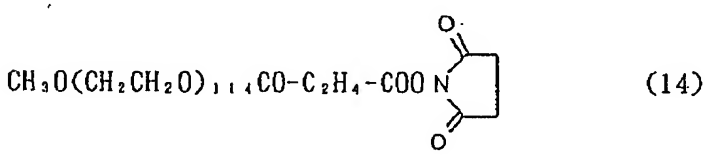
an antiserum obtained from a rabbit immunized with L-asparaginase, whereby the binding ability with antibody (antigenicity) was determined.

[0077]

Comparative Test Example 1

To 2 ml of 0.1M borate buffer (pH 10) containing 10 mg of L-asparaginase was added the compound of the formula (14):

[0078]



(14)

[0079]

in an amount of 11 molar equivalents to an amino group in the asparaginase molecule, followed by 1 hour of reaction at 37°C. Purification was conducted in a usual manner to obtain a modified asparaginase as a white powder. The molecular weight thereof was 420,000 and, as a result of analysis of the amino group, 54 groups were combined, so that the molecular weight was almost coincident with the total value of molecular weight of the incorporated part of 54 x 5200 = ca. 280000 and the molecular weight of asparaginase of 134000. The binding ability thereof with antibody was decreased to 35%. It maintained enzymatic

activity at the rate of 15% in Method A and 22% in Method B. These results are shown in Table 4.

[0080]

In this connection, the number of amino groups combined in the asparaginase molecule was measured with trinitrobenzenesulfonic acid. Moreover, the enzymatic activity was measured by a method of spectroscopically measuring a changed amount of NAD+ involved in malic acid formation using L-glutamic acid-oxalacetic acid transaminase (Method A) and by a method of coloring, with ferric chloride, the formation of aspartate hydroxamate by the above enzyme in the co-presence of aspartic acid and hydroxyamine (Method B). Furthermore, the antigenicity was measured by a method of measuring an amount of precipitate formed by an antigen-antibody reaction using an antiserum obtained from a rabbit immunized with L-asparaginase, whereby the binding ability with the antibody (antigenicity) was determined.

[0081]

[Table 4]

	PEG derivative (Mw)	Number of amino groups combined[a)]	Enzymatic activity		Binding ability with antibody
			Method A	Method B	
Test Example 1	5,417	52	37	41	0
Comparative Test Example 1	5,200	54	15	22	35

a): Number of the amino groups combined with the compound

among the amino groups (92 groups) in the asparaginase molecule.

[0082]

[Advantage of the Invention]

The compound of the present invention has a carboxyl group or an activated carboxyl group at the terminal of γ-position and hence can easily react with an amino group or hydroxyl group of polypeptides, physiologically active proteins, enzymes, and the like. Also, there is provided a carboxyl group-containing polyoxyalkylene compound which can achieve performance characteristics such as reduction of antigenicity of the above substances, stabilization thereof, and extension of the residence time in a body (blood) thereof, shows a low toxicity, and results in small formation of by-products.

[Brief Description of the Drawings]

[Fig. 1]

The drawing shows an infrared absorption spectrum of the compound obtained in Production Example 1.

[Fig. 2]

The drawing shows a differential and integral molecular weight distribution curve on GPC of the compound obtained in Production Example 1.

[Fig. 3]

The drawing shows a differential and integral molecular weight distribution curve on GPC of the compound obtained in Production Example 2.

[Fig. 4]

The drawing shows a differential and integral molecular weight distribution curve on GPC of the compound obtained in Production Example 3.

[Fig. 5]

The drawing shows an infrared absorption spectrum of the compound obtained in Example 1.

[Fig. 6]

The drawing shows an infrared absorption spectrum of the compound obtained in Example 4.

Fig. 1

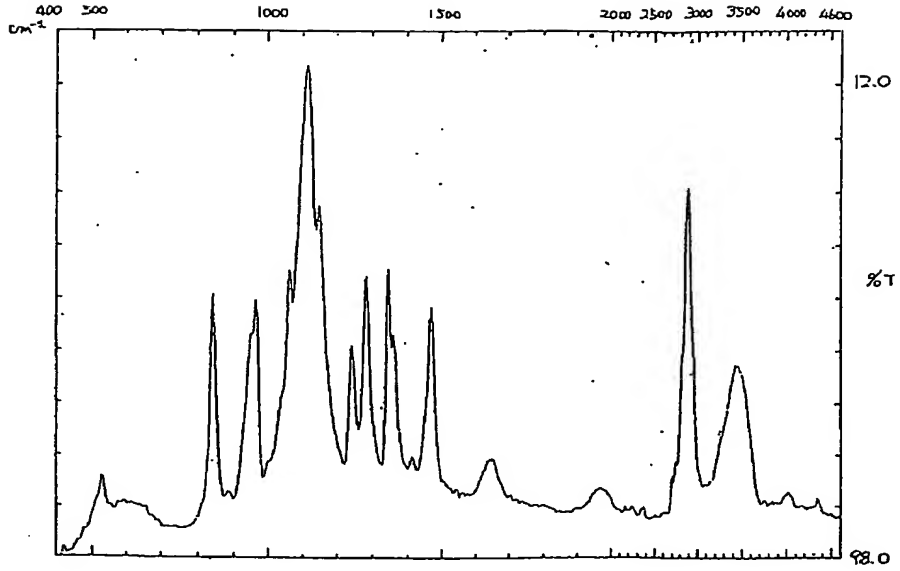


Fig. 2

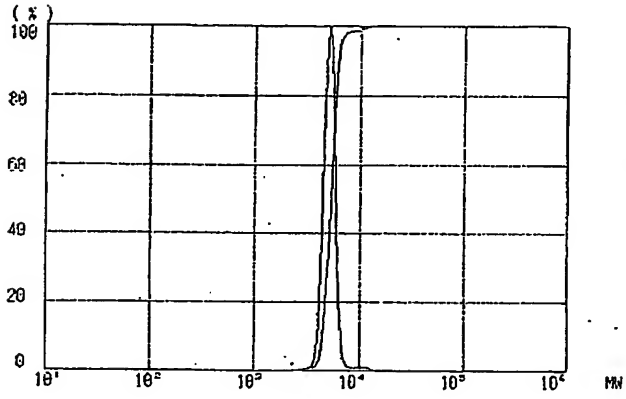


Fig. 3

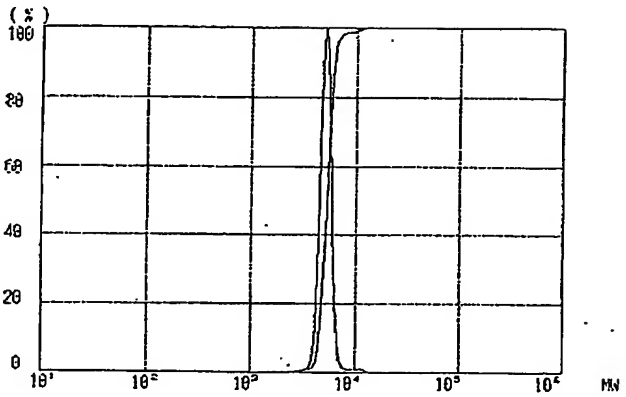


Fig. 4

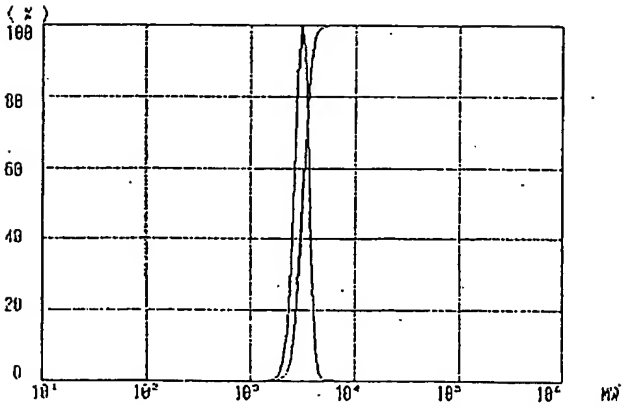


Fig. 5

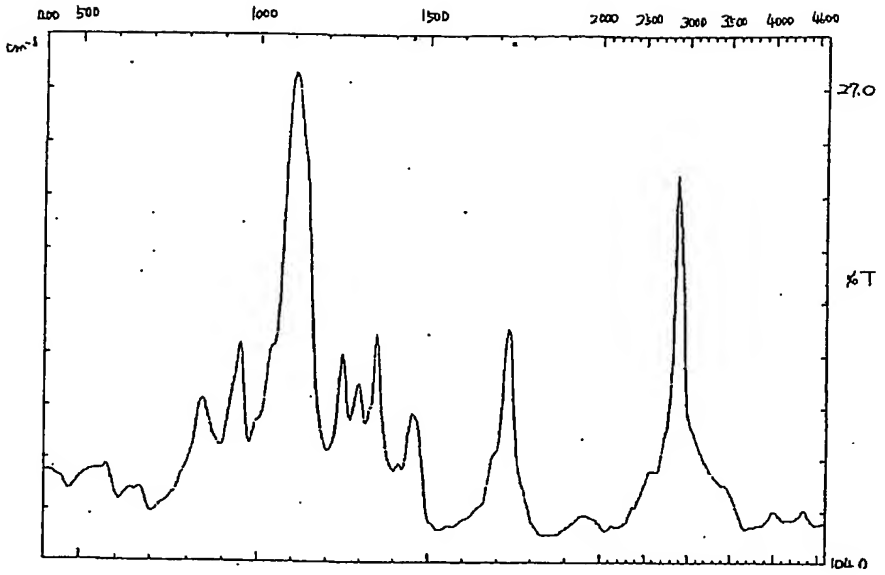
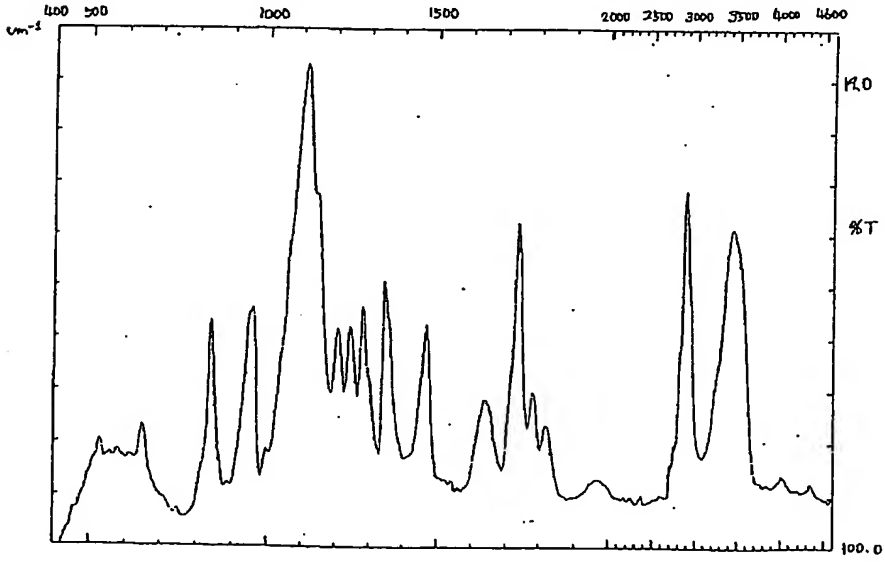

400 500 1000 1500 2000 2500 3000 3500 4000 4600
cm-1
27.0
%T
100.0

Fig. 6


400 500 1000 1500 2000 2500 3000 3500 4000 4600
cm-1
19.0
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